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(54) ENZYME SYSTEM COMPRISING AN ENZYME BONDED IN A POROUS MATRIX

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See application file for complete search history.

(56) References Cited

U.S. PATENT DOCUMENTS

4,539,294 A	9/1985	Metcalfe et al 435/180
5,077,210 A	12/1991	Eigler et al 435/176
5,645,891 A	7/1997	Liu et al 427/376.2
5,705,813 A	1/1998	Apffel
5,843,767 A	12/1998	Beattie 435/287.1
5,922,299 A	7/1999	Bruinsma et al 423/335
5,951,962 A	9/1999	Muller et al 423/702
6,326,326 B1	12/2001	Feng et al 502/62
6,635,226 B1	10/2003	Tso et al 422/129
6,696,258 B1*	2/2004	Wei et al 435/7.2

OTHER PUBLICATIONS

Diaz et al., Journal of Molecular Catalysis B: Enzymatic 2 (1996), pp. 115-126.*

Gimon-Kinsel et al., Studies in Surface Science and Catalysis, vol. 117, 1998, pp. 373-380.*

X Feng et al., "Self-Assembled Monolayers on Messoporous Supports (SAMMS) for RCRA Metal Removal", p. 5.15-5.20. PNNL.

A K Singh et al., "Development of Sensor for Direct Detection of Organopharphates. Part I: Immobilization, Characterization and Stabilization of Acetylcholinesterase and Organophosphate Hydrolase on Silica Supports", p. 1-11. 1999.

PL Havens et al., "Reusable Immobilized Enzyme/Polyurethane Sponge for Removal and Detoxification of Localized Organophosphate Pesticide Spills", p. 2254-2258. 1993.

P Mulchandani et al., "Biosensor for Direct Determination of Organophosphate Nerve Agents. I. Potentiometric Enzyme Electrode", p. 77-85. 1999.

M Huckel et al., "Porous Zirconia: A New Support Material for Enzyme Immobilization", p. 165-179. 1996.

X Feng et al., "Functionalized Monolayers on Ordered Mesoporous Supports", p. 923-926. 1997.

PCT Written Opinion based on PCT/US02/05755, mailed Jun. 14, 2005.

PCT Search Report, mailed Sep. 25, 2003, PCT/US 02/05755.

Kanno et al., "Enhanced Enzymatic Reactions in a Microchannel Reactor," Aust. J. Chem.,55, 687-690, (2002).

Lei et al., "Entrapping Enzyme in a Functionalized Nanoporous Support," J. Am. Chem. Soc., 124, 11242-11243, (2002).

"Applications for Enzymatic Microreactors," web page (1999).

Humphrey et al., "Enzyme immobilisation using SBA-15 mesoporous molecular sieves with functionalized surfaces," J. Molec. Catal. B: Enzymatic 15, 81-92 (2001).

Gavrilidis et al., "Technology and Applications of Microengineered Reactors," Trans IchemE, 80, part A, 3-30 (Jan. 2002).

Takahashi et al., "Immobilized enzymes in ordered mesoporous silica materials and improvement of their stability and catalytic activity in an organic solvent," Microporous and Mesoporous Materials, 44-45, 755-762 (2001).

Washmon-Kriel et al., "Cytochrome c immobilization into mesoporous molecular sieves," J. Molec. Catal. B:Enzymatic 10, 453-469 (2000).

Humphrey et al., "Enzyme Immobilization using siliceous mesoporous molecular sieves," Microporous and Mesoporous Materials, 44-45, 763-768 (2001).

Han et al., "Mesoporous Silicate Sequestration and release of Proteins," J. Am. Chem. Soc. 121, 9897-9898 (1999).

Ackerman et al., "Enzymatic Microreactors," AIChE.

Liu et al., "Molecular Assembly in Ordered Mesoporosity: A New Class of Highly Functional Nanoscale Materials," J. Phys. Chem. A 2000, 104, 8328-8339 (Sep. 2000).

Richins et al., "Expression, Immobilization, and Enzymatic Characterization of Cellulose-Binding Domain-Organophosphorus Hydrolase Fusion Enzymes," Biotechnology and Bioeng., 69, 591-596 (Sep. 2000).

Takahashi et al., "Catalytic Activity in Organic Solvents and Stability of Immobilized Enzymes Depend on the Pore Size and Surface Characteristics of Mesoporous Silica," Chem. Mater. 12, 3301-3305 (Nov. 2000).

Taupin et al., "Surexpression Dans *E. coli* Et Purification D'une Enzyme Bacterienne Degradant Les Organophosphores La Phosphotriesterase De Flavobacterium SP," Trav. Scient., 177-178 (1997).

Yan et al., "Recent Progress on Immobilization of Enzymes on Molecular Sieves for Reactions in Organic Solvents," Applied Biochem. And Biotech., 113-129 (2002).

* cited by examiner

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(57) ABSTRACT

A protein system is described in which a protein is bound within a matrix material that has pores that are sized to achieve excellent properties such as: activity, protein density, and stability. In a preferred embodiment, the pore sizes range from 50 to 400 Å. One protein that has demonstrated surprisingly good results in this system is OPH. This protein is known to degrade organophosphorus compounds such as are found in chemical weapons and pesticides. Novel methods of forming the protein system and methods of making OPH are also described.